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To: C.W. Jameson
National Toxicology Program, Report on Carcinogens
MD EC-14
P.O. Box 12233
Research Triangle Park, NC 27709 (fax 919/541-2242)

From: Richard Clapp, D.Sc., associate professor, Department of Environmental Health, Boston University, Boston

Samuel Epstein, M.D., professor of environmental medicine, School of Public Health, University of Illinois Medical Center, Chicago

Emmanual Farber, M.D., Ph.D., professor of pathology, Jefferson Medical College, Philadelphia; chairman of the National Academy of Sciences' 1978 Panel I: Saccharin and its Impurities

Michael F. Jacobson, Ph.D., executive director, Center for Science in the Public Interest, Washington, DC

Marvin S. Legator , Ph.D., professor of toxicology, The University of Texas Medical Branch at Galveston

William Lijinsky, Ph.D., former director, chemical carcinogenesis program, Frederick Cancer Research Center, Bethesda

Umberto Saffiotti, Ph.D., former chief, laboratory of experimental pathology, National Cancer Institute, Bethesda

The NTP recently requested public comment on the petition to delist saccharin from the upcoming 9th Report on Carcinogens. The NTP request was in response to conflicting recommendations from three scientific review groups on the scientific merits of the petition.[1] An NIEHS staff review and an NTP interagency group recommended delisting saccharin, but a scientific peer review by the NTP Board of Scientific Counselors voted to retain saccharin as "reasonably anticipated to be a human carcinogen" in the next Report on Carcinogens.

Four critical areas were identified for public comment, as (1) adequacy of existing epidemiologic data; (2) levels of human exposure, especially women and children; (3) mechanism of urinary bladder tumor formation in male rats versus female rats, and male and female mice; and (4) test data with laboratory animals showing tumor formation in target sites other than the urinary bladder. This letter is a response to these issues and explains why we believe that the scientific evidence does not support

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[1]Federal Register 63(53); 13418-13421, March 19, 1998, requesting comments by May 15, 1998.

delisting saccharin from the NTP Report on Carcinogens.

1. Adequacy of epidemiologic studies on saccharin

a. Numerous epidemiologic studies do not show an *overall* elevated risk of bladder cancer in humans consuming artificial sweeteners including saccharin. But several studies show *significant and consistent* associations in some subgroups, suggesting that epidemiologic data cannot reasonably be used to conclude that saccharin is safe (Howe et al. 1979; Hoover and Strasser 1980; Cartwright et al. 1981; Morrison et al. 1982; Mommsen et al. 1983; Sturgeon et al. 1994).[2]

b. The largest epidemiologic study (by nearly an order of magnitude) is compelling because it reported an increased risk of bladder cancer in non-smoking "low-risk" women and smoking "high-risk" men - two subgroups selected *a priori* as being of special interest (Hoover and Strasser 1980). The authors of this study suggested that elevated risks in smoking men might reflect *potentiation* of carcinogenic risk by artificial sweeteners.

c. The International Agency for Research on Cancer (IARC) commented that since the two subgroups in the Hoover and Strasser study were considered a priori as worthy of special attention on the basis of hypotheses generated from animal studies, the findings raise the possibility that saccharin may act as a weak carcinogen and/or promoter (IARC 1982).

d. IARC reported on two other case-control studies showing increased risk of bladder cancer in certain subgroups. One, conducted simultaneously in Japan, the US, and the UK, showed elevated relative risks for US women and non-smokers. The other, conducted in West Yorkshire UK found elevated relative risk with non-smokers (IARC 1987).

e. For the reasons outlined below, existing epidemiologic studies must be seen as revealing the *minimum* association between saccharin consumption and cancer. The lack of a *strong* association (high relative risk) is expected, based on animal studies. Also, several unavoidable methodological issues will weaken true positive associations.

- Animal studies show saccharin is only a weak carcinogen (IARC 1980). Weak carcinogens are inherently difficult to detect with epidemiologic studies.
- Many epidemiologic studies that did not detect increased risk were based on small numbers of subjects and had limited power for detecting small

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[2]References in parenthesis are taken from "Draft RC Background Document for Saccharin, September 25, 1997," prepared for the Board of Scientific Counselors, Report on Carcinogens Subcommittee, NTP, October 30 & 31, 1997. References that were not included in that review are given fully as footnotes.

increased risk from saccharin consumption.

- Significant exposure misclassification is likely since existing studies measured exposure to all artificial sweeteners including saccharin and cyclamates -- weakening any true associations (IARC 1980).
- Many studies lumped occasional consumers of artificial sweeteners with heavy consumers, thereby diminishing the power to detect an association.
- If saccharin acts primarily a cancer promoter/co-carcinogen in humans, then epidemiologic studies might have difficulty showing consistent effects because different populations intrinsically have large differences in exposures to cancer *initiators.*
- Animal studies show tumors at sites *other* than the bladder. Yet epidemiologic studies generally focus *exclusively* on bladder cancer and likely would have overlooked cancer at other sites. Possible promotion and co-carcinogenic effects at sites *other* than the bladder are difficult to investigate in humans.
- Bladder cancer has a latency of twenty to thirty years or more, but most studies focus on cases diagnosed before the early 1980s. No human cases would have had substantial exposure *in utero* or as a child, although animal data strongly indicates that those are the most important exposure periods.
- Nevertheless, epidemiologic studies *do* show elevated risk ratios in groups with long use (e.g., 30 to 40 years) of saccharin.

Elevated risk ratios for bladder cancer in humans with long (30 to 40 years) use of saccharin

Subjects	*Risk ratio*	*Source*
Non-smoking males in UK or Japan	1.6	Morrison et al. 1982
Non-smoking males in UK	2.2 (1.3-3.8)	Cartwright et al. 1981
Non-smoking females in UK	1.5 (0.8-3.2)	Cartwright et al. 1981

f. IARC concluded in 1987 that "The epidemiologic data taken as a whole cannot with confidence exclude a small increase in risk but provide no clear evidence that artificial sweeteners cause bladder cancer in humans." This is hardly an endorsement of saccharin's safety.

2. Human exposure and safety factors

a. Sodium saccharin is used in significant quantities on a daily basis by many US consumers including young children and pregnant woman. Human consumption levels would likely considerably increase if saccharin were delisted from the Report on Carcinogens. Comparison of dose levels in animal

studies that are positive for bladder tumorigenicity or cell proliferation to estimated human intake, especially for children, show that they are not as outrageously different as popular belief has suggested.

Safety factors comparing estimated daily human exposure levels to daily dose NOEL exposure for rat bladder tumorigenicity and rat bladder cell proliferation (comparing mg/kg doses)[1]			
	Safety factor for a 1-year old child	**Safety factor for a 7-year old child**	**Safety factor for a 23-year old woman**
Comparing human exposure to NOEL for rat bladder tumors	50	25	50
Comparing human exposure to NOEL for rat bladder cell proliferation	5	2.5	5
[1]Data for daily human exposure and rat bladder cancer and cell proliferation NOELs taken from Hooper, Principal reviewer on saccharin, meeting of the Board of Scientific Counselors, Report on Carcinogens Subcommittee, NTP, October 30 & 31, 1997.			

b. In another estimate using default EPA assumptions to compare human and laboratory animal saccharin consumption, the observed NOEL dose causing bladder tumors in rats (1 percent in the diet) is approximately 20 to 28 times greater than the average human adult consumption (based on the 1977 USDA survey of saccharin consumption (NTP 1997, p 2-7).[3]

c. Comparison of 1977 US human consumption figures to exposures causing tumorigenicity and cell proliferation in laboratory animals is relevant to estimating *current* human risks. US per capita consumption of saccharin has scarcely changed over the last three decades. In 1970 per capita consumption was 5.8 lbs and in 1991 it was 7.3 lbs. It reached a peak of 10.0 lbs in 1984, and was at it's lowest level of 5.1 lbs from 1971 to 1973.[4]

3. Mechanism of urinary bladder formation in male rats versus other test animals

a. The proposal to delist saccharin is based on a hypothesis that saccharin causes cancer only at high doses in the male rat bladder by formation of bladder precipitates due to a combination of events including an increase in urine pH and sodium ion concentration from high doses of sodium saccharin, and the unique presence of high concentrations of the protein alpha 2u-globulin

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[3] Pers. com. Robert Maronpot, NIEHS, October 31, 1997. Assumptions: the rat weighs 400g, and eats 50g diet/day, which contains 1 percent sodium saccharin, and the human adult weighs 70 kg. EPA's default assumption compares (body weight)$^{3/4}$.

[4] USDA, Food consumption, prices, and expenditures, 1970-95. Statistical bulletin no. 939, August 1997, p. 71.

in male rat urine. Unique to the male rat, these precipitates (containing alpha 2u-globulin, calcium phosphate and silicates and other inorganic salts), could enter bladder epithelial cells and cause focal necrosis through physical irritation, followed by cytotoxicity with regenerative hyperplasia, and eventually tumorigenesis. Although several studies show that the male rat is more sensitive to bladder cancer from saccharin, the bladder precipitates hypothesis leaves unexplained the positive data reported in numerous studies in female rats, and in female and male mice.

- Two 2-generation studies showed non-statistically significant increases in urinary bladder cancer in saccharin-treated *female* rats, along with statistically significant positive increases in urinary bladder cancer in male rats (Taylor 1989; Arnold 1980).
- According to the 1997 NTP review, four mouse studies show positive carcinogenicity (Allen et al. 1957; Bryan et al. 1970; Prasad and Rai 1986; and Frederick et al. 1989). Four mouse studies were negative. Three additional mouse studies were not included in the NTP review and are discussed in section 4, below.
- Sodium saccharin is reported to induce urinary bladder tumors in male rats only if it is administered before the rat is 35 days old (Cohen et al. 1995b). Yet biosynthesis of the critical factor alpha 2u-globulin in male rat does not begin until 35 to 40 days of age and is undetectable before that (NTP 1997; Roy et al. 1983; Neuhaus and Flory 1978). "While this does not necessarily preclude a role for alpha 2u-globulin in sodium saccharin carcinogenesis, it does raise some doubts" (NTP 1997, p. 7-15).
- Some studies show that saccharin exposure *does not* increase urinary pH and salt concentrations (NTP 1997 table 7-2, p. 7-18). Others note that "[a]lthough a 7.5% sodium saccharin diet increased the concentration of sodium ion approximately 3-fold [in urine compared to typical rat chow], this concentration scarcely represents a large increase from the usual daily dietary intake of sodium ion" (NTP 1997, p. 7-7).
- With regard to the bladder precipitates hypothesis for saccharin carcinogenicity, the characteristics and conditions described above as required for bladder carcinogenicity have been extensively studied in male rats but far less so in female rats (NTP 1997).
- The male rat data used to support the bladder precipitates hypothesis show only an *association* between bladder precipitates and cancer. Associations are not sufficient basis to conclude *causality*.
- Saccharin appears to be the only investigated example that associates bladder cancer with bladder precipitates. A causal relationship between precipitates and bladder cancer is at most a possibility in male rats, but causality has not been proven. Since saccharin causes bladder cancer in

female rats and in mice without precipitates, the *general* role of precipitates in humans and laboratory animals is questionable.

b. Several studies show that saccharin is a cancer *promoter* and a *co-carcinogen,* and causes *cell proliferation* in the urinary bladder of both sexes of several strains of male and female rats. Saccharin causes statistically significant dose-related reduced latency and/or increased incidence of urinary bladder cancer when combined with various bladder cancer initiators, described below. These results are not explained by a bladder precipitate mechanism.

- Sodium saccharin induces cell proliferation and bladder tumors in male *and* female rats after treatment with urinary bladder initiators (BBN or MNU) *without* involving bladder precipitates (Nakanishi et al. 1980a; Hicks et al. 1973 and 1975; Hicks and Chowaniec 1977; Mohr et al. 1978; West et al. 1986).
- Sodium saccharin promotes hyperplastic and neoplastic activity in the rat bladder with sub-carcinogenic doses of MNU, FANFT, BBN, and AAF in both male and female rats without involvement of bladder precipitates (Nakanishi et al. 1980a; Ershoff and Baja 1974; Fukushima et al. 1981; Hooson et al. 1980).
- Sodium saccharin alone promotes cell proliferation (hyperplasia) in female and male rat bladder *without* involvement of bladder precipitates (Lessel 1971; Fukushima and Cohen 1980; Hooson et al. 1980; Taylor et al. 1980; Masui et al. 1988 abstr.; Garland et al. 1989b; Cohen et al. 1995b).
- The NOEL reported for male rat bladder cell proliferation (0.1 percent dietary) is 1/10 the NOEL for male rat bladder tumorigenesis (1.0 percent dietary) (Murasaki and Cohen 1981; Schoenig et al. 1985). If cell proliferation is a key step in tumorigenesis, tumors will occur at less than 1 percent exposure at an incidence rate greater than zero. Thus, a 1 percent exposure may be a statistical rather than physiological NOEL.
- Tumor promoter, co-carcinogen, and cell proliferation effects of saccharin with other carcinogens have not been extensively tested with female compared to male rats, but when examined females appear to be as sensitive as males.
- Promoter/co-carcinogenicity appears to lack a mechanistic paradigm that would be comparable to the mechanistic paradigm for initiators.
- Saccharin could give the NTP the opportunity to tackle the larger issue of evaluating the human cancer potential of chemicals with clear-cut promoter/co-carcinogenic activity. This is important because humans are exposed to an array of carcinogens from food, the environment, and the workplace. From a public health perspective, promoters and co-

carcinogens could be as important as initiators.

c. Saccharin is at least weakly genotoxic in some in vivo and in vitro assays. Tests show it causes dominant lethal mutations in mice, which is a relatively insensitive test for genotoxicity.[5]

d. The American Cancer Society estimated 54,500 new cases of bladder cancer in 1997. Researchers have limited understanding of bladder cancer etiology in animals, and human children and adults. Basing public policy on a claim that one mechanism explains *all* carcinogenicity of saccharin exaggerates scientists understanding of this widespread disease.

4. Saccharin causes tumors in laboratory animals at sites other than the bladder

a. Numerous animal studies strongly suggest that laboratory animals fed saccharin develop tumors at target sites other than the bladder. Similarly, bladder tumors are reported in laboratory animals other than the male rat.

b. Some animal studies focused specifically on bladder carcinogenicity, and tumors at *other* sites could have been overlooked.

c. Increases in thyroid and Harderian gland tumors (the latter were significant and dose-related) are reported in separate bioassays of sodium saccharin in mice, obviously without the formation of bladder precipitates (Prasad & Rai 1986; Frederick 1989).

d. The Wisconsin Alumni Research Foundation (WARF) Institute study showed a dose-dependent and nearly statistically significant increase in the incidences of malignant tumors of the ovary and uterus of female rats at the highest dose of saccharin.[6] Both male and female weanling rats were fed 0, 0.05, 0.5, and 5 percent sodium saccharin for 100 weeks.

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[5] Rao, M.S. and Qureshi, A.B. Induction of dominant lethals in mice by sodium saccharin. *Indian J. Med. Res.* **60**, 599-603, 1972; Sram, R.J. and Zudova, Z. Mutagenicity studies of saccharin in mice. *Bull. Env. Contam. Toxicol.* **12**, 186-192, 1974; Masubuchi, M.A., Takahashi, O., et al. The mutagenicity of sodium saccharin (S-Na) I. Dominant-lethal test. *Mut. Res.* (Abstract 17) **54**, 218-219, 1978; and Tezebwala, B.U and Gothoskar, S.V. Preliminary studies on mutagenicity of saccharin by induction of dominant lethals. *Indian J. Cancer* **14**, 232-234, 1977. These studies were not included in the 1997 NTE review.

[6] WARF Institute, Madison, WI. Long-term saccharin feeding in rats. Final report to the International Sugar Foundation, March 26, 1973. Reviewed in Reuber, M.D., Carcinogenicity of saccharin. *Env. Health Persp.* **25**, 173-200, 1978.

Malignant tumors in uterus and ovary of female rats ingesting saccharin, WARF study		
Dose (percent in diet)	Number of mice with tumors/total number	p (Fisher exact test)
0	0/17	--
0.05	1/17[a]	0.50
0.5	2/15[a]	0.21
5	4/20[b]	0.07
[a] Squamous cell carcinomas of the uterus. [b] Carcinomas of the ovary.		

e. The National Institute of Hygienic Sciences study reported female mice showed a significant and dose related increase in ovary tumors.[7] Both male and female mice were fed saccharin at 0, 0.2, 1, or 5 percent for 21 months.

Ovary tumors in female mice ingesting saccharin, National Institute of Hygienic Sciences study		
Dose (percent in diet)	Number of mice with tumors/total number	p (Fisher exact test)
0	0/14	--
0.2	3/18	0.165
1.0	7/11	0.001
5.0	6/12	0.004

f. Bio-Research Consultants carried out two identical studies that found an increased incidence of vascular tumors in male mice.[8] Both male and female mice were fed 0, 1, or 5 percent saccharin for 104 weeks or longer. Review of this data by the US Congressional Office of Technology Assessment

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[7] The National Institute of Hygienic Sciences, Department of Toxicology, Tokyo, Japan. Chronic toxicity study of sodium saccharin (undated). Data from Reuber review.

[8] Bio-Research Consultants, Inc., Cambridge, MA. Final report, studies on saccharin and cyclamate, May 31, 1973. Data from Reuber review.

concluded that it supports "an association between saccharin and an increase in total and vascular tumors in males; furthermore, the number of vascular tumors was increased in saccharin-fed mice."[9]

Vascular tumors in male mice, Bio-Research Consultants study (combining both groups)		
Dose (percent in diet)	Number of mice with tumors/total numbers	p (Fisher exact test)
0	1/19	--
1	2/29	0.64
5	10/34	0.03

g. The last three studies, which were not included in NTP's 1997 literature review, suggest that saccharin causes cancer in laboratory animals at sites other than the bladder of male rats.

The epidemiologic and toxicologic data described in this letter overwhelmingly supports the continued listing of saccharin as is in the Report on Carcinogens, as *reasonably anticipated to be a human carcinogen.* For these reasons we believe that the NTP must reject the proposal to delist saccharin from the 9th edition of the Report on Carcinogens.

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[9] Office of Technology Assessment (OTA), "Cancer testing technology and saccharin." Oct. 1977, Appendix I, p. 76.